A comprehensive review on Tocilizumab in COVID-19 acute respiratory distress syndrome

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Abstract

Currently, the world is facing the pandemic of a novel strain of beta-coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Acute respiratory distress syndrome (ARDS) is the most devastating complication of SARS-CoV-2. It was indicated that cytokine release syndrome (CRS) and dominantly IL-6 play a central role in the pathophysiology of ARDS related to the novel 2019 coronavirus disease (COVID-19). Despite the global emergency of the disease, at this time, there are no proven therapies for the management of the disease. Tocilizumab is a potential recombinant monoclonal antibody against IL-6 and currently is under investigation for the management of ARDS in patients with COVID-19. Given these points, we reviewed the current evidence regarding the potential therapeutic role of tocilizumab and its important clinical issues in the treatment of ARDS related to COVID-19.

Key words: SARS-CoV-2, COVID-19, ARDS, IL-6, CRS, tocilizumab, acute lung injury

Introduction

Coronaviruses are a large family of RNA viruses with a widely found in nature. Generally, coronaviruses are animal pathogens, but in humans, six types of coronaviruses were known to cause respiratory tract infections ranged from mild to severe disease. The outbreaks of severe acute respiratory syndrome coronavirus (SARS-CoV), in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV), in 2012 were two severe types of infections caused by beta-coronaviruses with 11-35% mortality. [1-3]

Late in December 2019, a novel strain of beta-coronavirus was recognized to cause a cluster of cases of acute pneumonia in Wuhan, Hubei province, China. Unlike with SARS-CoV and MERS-CoV, the novel virus has a lower rate of mortality, but a higher rate of transmissibility and infectivity that rapidly spread throughout the globe. The World Health Organization (WHO) declared a pandemic outbreak and named the disease as coronavirus disease 2019 (COVID-19). Along with, the International Committee on Taxonomy of Viruses (ICTV) called the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). At the time of writing this manuscript, globally more than 7,410,000 confirmed cases of COVID-19 with more than 418,000 deaths have been reported. [1-3]

Person to person spread is the main route of virus transmission, which occurs by respiratory droplets. Notably, inhaled aerosols are another proposed pathway for transmission of SARS-CoV-2. It is indicated that viable viruses could be detected in aerosols up to 3 hours after aerosolization. Transmission of the virus can take place with the contact of contaminated surfaces or objects with the eyes, nose, and mouth.[4, 5]

Classically, the well-known symptoms of COVID-19 include fever, cough, and shortness of breath. A recently pooled meta- analysis of 43 studies involving 3600 patients with COVID- 19, showed that the most common clinical manifestation of the disease was fever (83.3% [95% CI 78.4–87.7]), followed by cough (60.3% [54.2–66.3]), fatigue (38.0% [29.8–

46.5]), myalgia (28.5% [21.2–36.2]), increased septum production (26.9% [18.3–36.4]), chest pain (14.9% [4.9–28.4]), chill (15.0% [0.3–41.4]), headache (14 [9.9–18.6]), sore throat (12.3% [8.5–16.5]), dizziness (7.6% [0.0–23.5]), and diarrhea (8.4% [4.8–12.6]). A recent observational study of 2013 European patents with mild to moderated COVID-19 reported the loss of smell and taste dysfunction in 87% and 56% of patients, respectively. Accordingly, the Centers for Disease Control and Prevention (CDC) has added six new symptoms including new loss of taste or smell, muscle pain, headache chills, repeated shaking with chills, and sore throat to its list as other symptoms of COVID-19. [6-9]

Acute respiratory distress syndrome (ARDS) is the most devastating complication of SARS-CoV-2 with a higher death toll. In a meta-analysis study, ground-glass opacity (80% [67.3–90.4]), >3 affected lobes (57.3% [42.6–71.4]), fibrous stripes (25.9% [2.9–59.8]) were common chest computed tomography (CT) findings. Moreover, the incidence of ARDS and death were (15.7% [5.0–30.4]), (3.6% [1.1–7.2]), respectively. The incidence of ARSD following COVID-19 is higher in severe cases. For example, in a study of 52 patients with critically ill 67% (35 patients) developed ARDS with a higher mortality rate (32 patients died). [6, 8]

Cytokine release syndrome (CRS) in its severe form is a life-threatening acute systemic inflammatory syndrome characterized by multi-organ damage and fever. It has been indicated to have a central role in the development of ARDS following SARS-CoV-2 infection. Interleukin 6 (IL-6) is shown to play the key role in the COVID-19 induced CRS, and its elevated levels have been observed in these patients.[10-12] Despite the global emergency of the disease, currently, there are no proven therapies for the management of the disease. Most of the treatments are undergoing clinical trials and mainly include antiviral or anti-inflammatory medications or conservative therapies.

Tocilizumab, sarilumab, and siltuximab are commercially available IL-6 inhibitors. They are under investigation for the management of ARDS induced by COVID-19. Tocilizumab is a recombinant humanized monoclonal antibody that binds to both membrane-bound and soluble forms of the IL-6 receptor. Labeled indications for tocilizumab include rheumatoid arthritis (RA), systemic juvenile idiopathic arthritis (SJIA), polyarticular juvenile idiopathic arthritis (PJIA), giant cell arteritis (GCA), and CAR T-cell induced severe CRS. Furthermore, it is used in the treatment of severe CRS due to Bi-specific T-cell engager (BiTE) therapy. Sarilumab is a fully human immunoglobulin G1 (IgG1) monoclonal antibody that binds to both soluble and membrane-bound IL-6 receptor with high affinity and is Food and Drug Administration (FDA) approved for the treatment of Rheumatoid Arthritis (RA). Siltuximab is a chimeric, human-murine immunoglobulin monoclonal bind directly to human IL-6 to neutralize it. The only labeled indication of siltuximab is for the treatment of Castleman disease. Tocilizumab is the first marketed IL-6 blocker that has been widely used in the treatment of patients with inflammatory diseases. Importantly, it is the only monoclonal antibody drug with FDA approval for the treatment of CAR T-cell induced CRS. [1-6, 12, 13]

Based on the potential role of tocilizumab in the management of CRS, the main role of IL-6 in CRS, and the marked role of CRS in the pathophysiology of ARDS of SARS-CoV-2, we aimed to review the current evidence concerning to safety and efficacy of the use of tocilizumab in the management of ARDS in the patients with COVID-19.

Pathophysiology:

Mechanism of cell entry of SARS-CoV-2

The SARS-CoV-2 spike glycoprotein (S) binds the host cell surface via angiotensin-converting enzyme-2 (ACE-2) receptor allowing virus cell entry and replication. [14] It is indicated that SARS-CoV-2 recognizes the human ACE-2 receptor more efficiently than

SARS-CoV. Moreover, it has a strong binding affinity to the human ACE-2 receptor. [10] Expression of the ACE-2 receptor is found in the heart, kidney, endothelium, and intestine, with a higher ratio in pulmonary tissues.[115- 20] Evaluating normal lung tissue from eight adult donors showed that 83% of ACE-2-expressing cells were alveolar epithelial type II cells. Accordingly, these cells are a reservoir for SARS-CoV-2 invasion.[15]

SARS-CoV-2 induced lung injury

The pathological features of SARS-CoV-2 are similar to SARS-CoV and MERS-CoV infections.[21]Furthermore, the envelope proteins (E proteins) involved in the viral assembly of SARS-CoV-2 and SARS-CoV share 95% homology and mediate the host immune reaction to coronaviruses. [22,23] It is believed that the delayed type-I interferon (INF) response plays a role in the process of SARS-CoV infection. In the initial phase, the virus evades pattern-recognition receptors and antagonizes the type-I INF response in the airway and alveolar epithelial cells, which leads to rapid viral replication. However, plasmacytoid dendritic cells and macrophages' response to SARS-CoV leads to a strong but delayed type-I INF response as well as releasing other inflammatory cytokines. The activation of type-I INF signaling cascades attracts neutrophils, inflammatory monocyte-macrophages, dendritic cells, and natural killer (NK) cells to the lung and a cytokine-driven vicious cycle occurs. The uncontrollable proinflammatory cytokines production such as IL-6 leads to diffuse alveolar damage with epithelial and endothelial apoptosis, dysregulated coagulation, and pulmonary fibrinolysis.[24- 27]In some cases of SARS-CoV, it was showed that ARDS can take place independently from viral load suggesting the important role of inherent properties of the host immune system rather than viral virulence on tissue.[1, 5, 28]

The SARS-CoV-2 activates the immune system through binding to the alveolar epithelial cells and leads to the release of cytokines, mainly IL-6. Consequently, alveolar-capillary

permeability to fluid, proteins, and blood cells is increased and respiratory failure occurs. [29-31] Evaluating the immune system of patients with COVID-19 showed that activation of abnormal pathogenic T- cells lead to the production of a large number of cytokines importantly IL-6 as well as induction of an inflammatory storm. In addition to IL-6, higher plasma levels of other cytokines including, IL-2, IL-7, IL-10, tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-1 alpha (MIP-1α), granulocyte-colony stimulating factor (G-CSF), interferon-γ-inducible protein-10 (IP-10), and monocyte chemoattractant protein-1 (MCP-1) were observed in intensive care unit (ICU) patients. Furthermore, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) play the key roles in the inflammatory storm, which probably leads to pulmonary fibrosis and organ failure through impairment of gas exchange across the alveolar-capillary membrane. [10, 11,]

Analyzing peripheral blood samples indicated that T cells and monocytes in severe/critical COVID-19 patients are significantly lower than healthy patients. Moreover, inflammatory monocyte with CD14+CD16+ phenotype and high IL-6 expression as well as Pathogenic Th1 cells with high expression of GM-CSF and IFN-γ exist in both peripheral blood and biopsy samples at autopsy of COVID-19 patients. Indeed, these inflammatory monocytes and pathogenic T cells stimulate the immune system and cause end-organ damage. [32] Postmortem examination of a patient who died of confirmed infection with COVID-19 demonstrated a bilateral diffuse alveolar damage with cellular fibromyxoid exudates. Besides, mononuclear inflammatory lymphocytes were observed in both lungs. Moreover, the characteristic viral cytopathic changes such as multinucleated syncytial cells with atypical enlarged pneumocytes in the intra-alveolar spaces were observed.[33]

Interleukin 6 and cytokine release syndrome

IL-6 is a multi-functional cytokine and has an important role in acute inflammation.[34] It has an essential role in the differentiation of B cells and the production of antibodies.[35] IL-6 is a pro-inflammatory regulator of T-cells that induce cytotoxic T-lymphocyte activity, stimulates T-helper 17 cell lineage and function as well as the development of self-reactive pro-inflammatory CD4 T-cell response, and inhibits the induction of regulatory T-cell stimulate. [36-38] Also, IL-6 stimulates the differentiation of osteoclasts and angiogenesis.[39]

CRS is a severe and life-threatening acute systemic inflammatory syndrome characterized by multi-organ damage and fever that often takes place in the patients received immunotherapy or haploidentical allogeneic hematopoietic cell transplantation and associated with a sharp increase of inflammatory cytokine levels; however, it can occur via viral infections. Clinical manifestations can range from the flu-like syndrome to circulatory collapse, pulmonary edema, hypoxia, peripheral edema, hypotension, and multiorgan system failure. [40-43]

In the pathogenesis of T-cell engaging immunotherapy, released INF- γ by activated T-cells activates macrophages. Afterwards, the activated macrophages releases TNF-α, IL-6, and IL-10. Furthermore, serum levels of IL-8, IL-5, and IL-1 are elevated in CSR and are thought to contribute to its clinical manifestations. TNF-α is associated with flu-like symptoms as well as malaise, fever, diarrhea, lung damage, cardiomyopathy, and vascular leakage. INF-γ is associated with fatigue, dizziness, headache, fever, and chills; and IL-6 is associated cardiomyopathy, activate of the complement and coagulation cascades, cardiomyopathy, and disseminated intravascular coagulation. [44-48] In the CAR T-cell therapy associated CRS, IL-6 is considered to be a key driver symptom and its levels increase dramatically (more than 100-fold). No studies have been carried out to evaluate the effects of tocilizumab for CAR-T associated CRS; however, rapid clinical improvement in several patient cohorts led to rapid

FDA approval in August 2017. Also, tocilizumab is used in the treatment of severe CRS due to BiTE therapy. [49, 50]

IL-6 plays an important role in the inflammatory storm in patients with COVID-19, and combining antiviral with anti-inflammatory treatments should be considered. [51, 52] A study by Wang Wenjun et al. showed that all 11 critically ill patients had a significant increase of IL-6 as an early indicator of CRS like reactions in COVID-19-infected pneumonia. 72.7% of patients had CRS like characteristics such as fever, pulmonary inflammation, an increase of IL-6, and multi-organ dysfunction. [53] Another study in patients with COVID-19 found that high levels of IL-6 are associated with the severity of pneumonia.[54] Besides, a retrospective multicenter study of 150 patients with COVID-19 pneumonia showed elevated levels of inflammatory factors such as ferritin ($p<0.001$), and IL-6 ($p<0.0001$) in the blood are the predictors of the mortality outcome.[55] CAR T-cell induced CRS and role of IL-6 briefly are shown in Figure 1.

It is indicated that levels of cytokines including IL-6, IL-2, IL-1β, IL-8, IL-17, IFN-γ, TNF-α, IL-4, Interferon gamma-induced protein 10 (IP-10), MCP-1 are elevated in patients with COVID-19.[56] Consequently, in addition to IL-6, other inflammatory cytokines have a crucial role in the development of CRS. It was shown that total T cells, CD4+ T cells, CD8+ T, NK cell numbers were significantly lower in severe/critical COVID-19 compared with mild patients and healthy subjects. Also, features of cellular abnormality and exhaustion have been observed in T cells and NK cells. Besides, therapies that act more generally such as corticosteroids might reduce the immune system. These drugs suppress immune cells especially CD4 T and CD8 T cells. Therefore, some guidelines such as Massachusetts General Hospital on COVID-19 treatment recommended that systemic steroids should be avoided in patients with COVID-19, and it may be used for other reasons such as refractory septic shock. In addition, the national institution health treatment guidelines for COVID-19

recommend a low-dose of corticosteroids in patients with COVID-19 and refractory shock. A multi-center quasi-experimental study in 213 patients with moderate to severe COVID-19 showed that early administration of methylprednisolone (0.25 to .5 mg/kg) twice daily for 3 days is associated with clinical outcomes improvement. Indeed, the occurrence of the composite endpoint was significantly lower in the early corticosteroid received group compared to the non-treated group (34.9% vs. 54.3%, p=0.005). Moreover, a single-center retrospective cohort study was done in 463 patients with COVID-19 pneumonia to determine the role of steroids in in-hospital mortality. Results indicated that the survival rate was higher in patients who received glucocorticoids compared to those not received. Furthermore, no statistically significant difference was observed in the mortality rate between the initial regimens of methylprednisolone (1 mg/kg/day) or equivalent and pulses of glucocorticoid. In contrast to these reports, a meta-analysis pooling 11 reports of SARS-CoV, MERS-CoV, and SARS-CoV-2-infected patients indicated that corticosteroids delay virus clearance, increase of mechanical ventilation rate, prolong hospitalization, and have no significant effect on mortality. Therefore, considering the key role of IL-6 in COVID-19 induced CRS, suppression of IL-6 governed immune response using tocilizumab allows other immune responses to fight COVID-19 and protects against the harmful effects of hyperinflammation. [57-62] Screening for hyperinflammation is suggested in patients with COVID-19. For example, ferritin seems to the diagnostic hallmark of macrophage activation syndrome (MAS) and is elevated in a patient with severe COVID-19, especially in secondary hemophagocytic lymphohistiocytosis (HLH). [61] In a retrospective, multicenter cohort study of 191 patients with COVID-19, serum levels of ferritin are significantly higher in non-survivors compared with survivors throughout the clinical course (1435.3 mg/L (728.9–2000.0), 503.2 mg/L (264.0–921.5); P-value = <0.0001) and increase with disease deterioration. [64] Besides, the

mean serum level of ferritin in hemodialysis patients increased after infection with SARS-CoV-2 from 584±318 mg/L to 1446±1261 mg/L. [65]

Tocilizumab potential for use, efficacy in COVID-19 based on published data

Currently, data about the use of tocilizumab in COVID-19 are limited. Consequently, we are looking forward to revealing the results of ongoing trials to draw a conclusion. In this line, two cases of successful treatment of COVID-19 in patients with malignant comorbidities have been reported with tocilizumab. The first patient with multiple myeloma was treated with a single dose of intravenous tocilizumab (8 mg/kg) on day 9 of hospitalization. Before treatment with tocilizumab, he received 40 mg methylprednisolone for 4 days. Despite an improvement in breathing, chest tightness, and chest CT imaging didn't improve. After treatment of tocilizumab, serum level of the patient's IL-6 dropped from 122 pg/ml to 21 pg/ml on day 18, and both clinical symptoms and chest CT imaging improved. [66]Another COVID-19 patient with a recent diagnosis of metastatic sarcomatoid clear cell renal cell carcinoma received two doses of intravenous tocilizumab (8 mg/kg) with 8 hours interval on hospital day 8, and the patients improved thereafter and recovered (Table 1). [67]

The published papers lack a clear analytical approach and show poor methodological quality. A retrospective, single-center, case series was carried out in 21 Chinese patients with critical (19%) and severe (81%) COVID-19. Critical COVID-19 defined as requiring mechanical ventilation or organ support in ICU. Severe COVID-19 included patients with tachypnea and/or respiratory failure. The mean age of patients was 56.8 ± 16.5 years, and 85.7 % of them were male. The mean level of IL-6 was 132.4 ± 278.5 pg/mL. All patients received standard care including lopinavir, methylprednisolone, other symptom relievers, and oxygen therapy. In addition to standard care, all patients received a single dose of intravenous

tocilizumab 400mg, three patients received a second administration of tocilizumab 400mg with 12 hours interval. It is important to mention that seven days before treatment with tocilizumab, all of the patients had received routine treatment; however, no improvement had been observed in symptoms, hypoxemia, and CT images. Results showed immediate improvement of symptoms, CT opacity changes, and hypoxemia after tocilizumab administration. Patients' fever improved completely within 24 hours post-administration. Radiological improvement in ground-glass opacities took place in 91% of patients. Notably, blood and oxygenation results were reported in 19 patients. Mean C-reactive protein (CRP) level decreased from 75.1 ± 66.8 mg/mL to 2.72 ± 3.6 mg/mL on day 5 after treatment. Furthermore, oxygen saturations of patients improved statistically significant within five days after treatment. One patient no longer needed supplementary oxygen; 15 had decreased oxygen support; one began the ventilator weaning process, and two were extubated. Finally, 19 patients discharged, and 2 were in a stable condition in the hospital. [68] According to the National Health Commission of China, clinical classification of the COVID-19 is as follow: mild (slight clinical symptoms but no imaging presentations of pneumonia); moderate (fever, respiratory symptoms and pneumonia performance on chest X-ray or CT); severe (respiratory distress with respiratory rate > 30 times/minutes, or oxygen saturation at rest <93%, or atrial partial pressure of oxygen/fraction of inspiration O2 (PaO2/FiO2) ratio <300 mmHg (1 mmHg=0.133 kPa); critically severe (respiratory failure needs ventilation, or shock, or combined with other organ failure, patients need intensive care unit monitoring and treatment). [69] Another retrospective, single-center, case series was done in 15 Chinese patients with moderately ill (13.3%), seriously ill (40%), and critically ill (46.7%) COVID-19. The median age of patients was 73 years, with a male majority of 75%. Baseline comorbidities of patients included diabetes mellitus, hypertension, and previous cerebrovascular accident (CVA) in 27%, 60%, and 20%, respectively. All patients were

administered at least one dose of tocilizumab (80 to 600 mg) of either alone (47%) or in combination with methylprednisolone (53%). Notably, 33% of patients received subsequent doses of tocilizumab. By day seven post-treatment, 67% of patients were clinically stable, 13% had deterioration of their disease, and 20% died. The baseline levels of IL- 6 ranged from 16.4 to 627.1 pg/mL. A mild rise of 74.8 pg/mL (-0.8-175.6) in median IL-6 level was observed after tocilizumab administration in ten clinically stabilized patients, while the remaining 5 patients experienced a dramatic rise of 3581.2 pg/mL (591.9-4983.6); however, the CRP levels rapidly, and significantly dropped from 126.9 mg/L (10.7- 257.9) to 11.2 mg/L (0.02- 113.7) after tocilizumab administration ($P < .01$). It is in accordance with the fact that CRP is an appropriate surrogate marker for tocilizumab levels and IL-6 bioactivity. [70]

A prospective, multi-center, case series was carried out in 63 patients with severe COVID-19. The mean age of the patients was 62.0 ± 12.5 years (mean ± SD), and 88.8% were male. Patients with polymerase chain reaction-confirmed COVID-19, pulmonary involvement (oxygen saturation <93% or Pao2 / Fio2 < 300 mm Hg), and at least three of the following criteria: lactate dehydrogenase (LDH) > two times of normal upper limit; ferritin >1000 mg/L; D-dimer > 10 times of normal values; CRP > 10 times of normal values. Clinical and laboratory parameters were assessed at baseline, and during 14 days after treatment. Among 63 patients, 34 patients received intravenous tocilizumab (8 mg/kg), and 29 received subcutaneous tocilizumab (324 mg), according to the drug availability. In addition, all patients but one received an additional dose of the drug within 24 hours. The mortality rate was 11%, and there was no statistically significant difference between subcutaneous and intravenous administrations. At admission, 25 patients had a fever, which resolved in 24 patients within 24 hours of the treatment. Furthermore, PaO2/FiO2 (mmHg) improved during the follow-up period (baseline: 152±53; day 7: 283.73±115.9, day 14: 302.2±126, $p<0.05$).

Also, the levels of CRP, ferritin, D-dimer, and lymphocyte count improved; however, no significant change in LDH levels was observed. Notably, the mean baseline D-dimer level was predictor of death (HR 5.01; 95%CI 1.04-29.17). Finally, results showed that tocilizumab decreased chance of mortality within six days of treatment (HR 2.2 95%CI 1.3-6.7, $p<0.05$). [71] A prospective, single-center case series was carried out in 100 patients with severe COVID-19. The median age of patients was 62 years, with a male majority of 82%. The severity of respiratory disease was evaluated using the Brescia COVID-19 Respiratory Severity Scale (BCRSS). [72] It classifies the severity of patients according to the need for ventilator support and oxygen supplementation, providing a step-up therapeutic approach for anti-inflammatory and antiviral drug use. Baseline comorbidities of patients are hypertension (46%), obesity (31%), diabetes mellitus (17%), and cardiovascular disease (16%). Patients with neutropenia (<500 / mm^3), thrombocytopenia (<50000 / mm^3), suspected or confirmed bacterial infection, or active diverticulitis or gastrointestinal tract perforation were excluded. Patients received two intravenous tocilizumab (8 mg/kg) up to a maximum dose of 800 mg, with 12 hours interval. A third dose was optional according to the clinical response after 24 hours of the second dose. Finally, 87% and 13% of patients received two and three doses of tocilizumab, respectively. Also, all of the patients received a standard pharmacological protocol (antiviral drugs, antibiotic prophylaxis, hydroxychloroquine 400 mg/day, and dexamethasone 20 mg/day). Fifty-seven patients were treated with non-invasive ventilation (BCRSS = 3) in the general ward, of whom 63% died, 12% remained stable, and 23% worsened. Forty-three patients were treated in the ICU after tracheal intubation and mechanical ventilation (BCRSS > 3), of whom 74% improved, 2% remained stable, and 24% died. The clinical condition was improved or stabilized in 77% of patients, and 23% worsened. Finally, 20% of patients died. Also, lymphocyte count, CRP, fibrinogen, ferritin levels improved within 10 days after treatment. While IL-6, D-Dimer levels increased. Serum

levels of CRP dropped from 113 mg/L (45-169) to 2 mg/L (1-5), whereas serum levels of IL-6 increased from 41 pg/mL (10 -102) to 1812 (375-2600); median (1st Quartile - 3rd Quartile)). [72] Furthermore, a retrospective study was done in 171 non-critically ill COVID-19 patients. Patients received the standard protocol including antiviral drugs, hydroxychloroquine, azithromycin, low molecular weight heparin (in patients with risk factors for thrombosis), and methylprednisolone (in patients with disease progression to ARDS). Among 171 patients, 77 was given tocilizumab, and 94 was not. Indeed, patients with progressive respiratory failure, and lymphocyte count < 800/mm^3, or CRP ≥ 80 mg/L, or ferritin ≥800 mg/L were prescribed tocilizumab. The dose of tocilizumab was 400 mg for patients with ≤75 kg, and 600 mg for patients with >75 kg. Patients with a partial response received one or two additional administrations every 12 hours. Comparing outcomes of the groups revealed that patients in the tocilizumab group had significantly fewer ICU admissions and the need for invasive ventilation, compared to the control group (10.3% vs. 27.6%, P= 0.005; 0 vs. 13.8%, P=0.001). Also, the mortality rate (10.3%) was lower in patients receiving tocilizumab compared to other reports. Importantly, according to multivariable analyses (considering potential confounders), tocilizumab remained as a strong protective variable of ICU admission or death (OR: 0.03, CI 95%: 0.007-0.1, P=0.0001). Indeed, this non-randomized clinical trial showed the beneficial effects of tocilizumab (400 or 600 mg) administration in the early stages of an inflammatory storm. It is important to mention that clinical deterioration of COVID-19 and the development of ARDS is rapid, and timely identification and treatment are vital. Patients should be evaluated regarding the factors that predict the progression of the disease to complicated stages. [73] A systematic review and meta-analysis of 30 studies including 53,000 COVID-19 confirmed patients showed that the risk factors for poor prognosis prediction of early-stage patients are as follow: old age, male sex, presence of comorbidities including chronic kidney disease (CKD),

chronic obstructive pulmonary disease (COPD), cancer, hypertension, diabetes, and laboratory indicators such as lymphopenia, thrombocytopenia, elevated D-dimer, CRP, low-density lipoprotein (LDL), alanine aminotransferase (ALT) and creatine kinase (CK), and mainly IL-6 levels. Screening for hyperinflation as well as the prognostic factors identifying the severity of the disease is recommended for all patients with COVID-19. [74]

Numerous ongoing studies are carrying out to evaluate the efficacy and safety of tocilizumab in patients with COVID-19 (Table 2). Among these 24 studies, twelve are based in Europe, ten in China, and two in the USA. The study sample sizes range from 20 to 500 with a cumulative sample size of 4269. Eligibility criteria vary across studies. Several factors such as the severity of the disease, respiratory status, risk factors for progression, and levels of cytokines were considered. The route of administration was subcutaneous or intravenous. The dosages of tocilizumab were based on the weight of patients and/or fixed-dose ranging from 1 to 8 mg/kg and/or 80 to 800 mg in each administration. Considering clinical response and trial protocol, patients could receive one or two additional administrations of the medicine with intervals of 12 to 48 hours in some of the trials. The most common dosage was intravenous tocilizumab (8 mg/kg) up to 800 mg, which is similar to the FDA approved dose for CAR T-cell therapy induced CRS. The studies compare tocilizumab with usual care or other agents with a possible beneficial effect in COVID-19. Finally, there was significant heterogeneity in the primary endpoints of studies, such as mortality rate, resolution of fever at 24 hours, clinical improvement, biochemical response, oxygen saturation, need for mechanical ventilation, and change of Sequential Organ Failure Assessment (SOFA) score.

Base on China's National Health Commission recommendation, tocilizumab should be considered for the treatment of patients infected with COVID-19, with elevated IL-6 levels and serious lung damage.[75] The FDA has approved a randomized, double-blinded, placebo-controlled phase 3 trial called COVACTA to evaluate the efficacy and safety of intravenous

tocilizumab 8 mg/kg (up to the maximum dose of 800 mg per dose) in patients with COVID-19 pneumonia. In this study, patients are allowed to receive an additional dose based on their clinical conditions. The inclusion criteria are hospitalized patients with COVID-19 pneumonia according to WHO criteria chest X-ray or CT scan.[76] Moreover, the Italian Regulatory Agency (AIFA) has approved a multicenter study (TOCIVD-19) to evaluate the efficacy and safety of tocilizumab 8 mg/kg (up to a maximum of 800 mg per dose) in the treatment of COVID-19 pneumonia patients. Patients can receive second administration (same dose) after 12 hours. Notably, the study project includes a parallel observational cohort study, and a single-arm phase 2 study. The inclusion criteria were confirmed diagnosis of SARS-CoV-2 infection, and oxygen saturation ≤93% in ambient air. In addition, patients with intubation less than 24 hours before registration are enrolled in phase 2, and those with intubation more than 24 hours before registration in an observational cohort. Patients are evaluated regarding laboratory data (blood count, bilirubin, aspartate aminotransferase (AST), ALT, creatinine, prothrombin time (PT), partial thromboplastin time (PTT), LDH, D-dimer), atrial blood pressure, 12-lead electrocardiogram, vital signs, SOAF score, radiologic findings, a respiratory assistant during the study period. A one-month mortality rate is the primary endpoint of the study. The secondary endpoints of the study include the duration of hospitalization, evaluation of CRP and IL-6 levels in correlation with treatment outcomes, respiratory symptoms, time to invasive mechanical ventilation, definitive extubation, and independence from oxygen therapy, radiological response, trends of PaO2/FiO2 ratio, SOFA score, and lymphocyte count, and tocilizumab toxicity. The Italian guideline recommended the presence of at least one of the following criteria before tocilizumab administration; IL-6 levels >40 pg/mL (or D-dimer >1000 mg/L), PaO2/FiO2 ratio <300 mmHg, respiratory gas exchange rapid worsening. According to the University of Michigan recommendations, tocilizumab should be considered in COVID-19 patients with abnormalities in chest imaging,

rapid worsening of gas exchange requiring >6 L/min O2, laboratory parameters of CRS, need for supplemental O2 to maintain PaO2/FiO2 <300 mmHg or saturations oxygen <92%, and at least two laboratory abnormalities (lymphocyte count < 600/mm3, D-dimer > 1000 mg/L, ferritin >500 mg/L, LDH >250 units/L, or CRP >100 mg/L or >50 but doubled in past 48 hours). [77-79]

Because immune system antiviral activity is vital to recovering from SARS-CoV-2 infection, the pros and cons of using tocilizumab on these patients should be considered with caution. Based on animal studies, IL-6 plays an important role in the clearance of viruses as well as control of pulmonary inflammation. The clinicians should be considered the severity of viral load or replication status and hyperinflammation. The application of some tools to design the severity scale of COVID-19 with focusing on levels of CRP and IL-6, ferritin, platelet counts, leukocyte counts, erythrocyte counts, and sedimentation rate is useful to guide clinicians to start the treatment. Furthermore, the application of the HScore has been recommended to recognize COVID-19 patients at high risk for hyperinflammation. The HScore is used for the evaluation of secondary haemophagocytic lymphohistiocytosis considering laboratory and clinical parameters, including body temperature, organomegaly, hemophagocytosis on bone marrow aspirate, and signs of immunosuppression, and serum AST, triglycerides, fibrinogen, cytopaenias, and ferritin. Also, patients should be evaluated considering clinical manifestation and laboratory findings of CRS. As discussed earlier, the administration of tocilizumab as a selective instead of broad immunosuppressive drugs is recommended to avoid the suppression of the anti-viral activity of the immune system. Furthermore, the timing of treatment is important to decrease the adverse effects of tocilizumab; however, evidence regarding the proper timing of administration is not definitive. Besides, the true dose of tocilizumab currently is unknown and needs to be addressed by underway studies. More researches are needed to determine when and for which patients, tocilizumab should be

administrated. [80, 81] Based on recent data, tocilizumab can be considered in patients with extensive lung involvement, and severe or critical patients with high IL-6 levels. The intravenous tocilizumab should be diluted to 100 ml with 0.9% normal saline, and the infusion time is at least one hour. The maximum dose should not exceed 800 mg intravenously. If clinical improvement does not take place after the first dose, one additional dose may be considered within at least 12 hours; however, up to two additional doses have been used in some studies. The recommended dose of tocilizumab in CAR-T cell therapy-induced CRS is 8 mg/kg (≥30 kg) and 12 mg/kg (<30 kg) up to 800 mg in each administration), intravenously with at least 8 hours interval up to four doses. In the study by Moreno-García et al., [73]one to three administration of lower doses of tocilizumab (400 mg or 600 mg) in the early stages of COVID-19 induced cytokine storm showed the beneficial effects in decreasing ICU admissions and death. Consequently, recognizing patients with risk factors for disease progression is useful. Importantly, in similar clinical conditions such as CAR-T cell and BiTE therapies induced CRS, subcutaneous tocilizumab has not been recommended. Besides, in chronic conditions such as RA, the maximum dose of subcutaneous tocilizumab is 162 mg. Notably, in the prospective case series by F Apra, [71] patients were given subcutaneous (324 mg) or intravenous (8 mg/kg) tocilizumab in COVID-19 induced CRS, and the mortality rate was not statistically significant between these two routes of drug administration. Taken together, based on limited data, tocilizumab is effective for patients with severe and critical COVID-19 related ARDS and needs to be addressed by ongoing clinical trials.

Tocilizumab safety and potential for toxicity

Adverse reactions of chronic administration of tocilizumab have been assessed in patients with rheumatologic diseases such as RA, and giant cell arteritis. In a double-blind,

randomized, placebo-controlled, parallel-group phase III study, 623 patients with RA were randomized to receive intravenously tocilizumab 8 mg/kg (n=205), tocilizumab 4 mg/kg (214), or placebo (204) every 4 weeks, with fixed doses of methotrexate. The results showed serious infections, as the most common serious adverse events, were observed in six, three, and two patients, respectively.[82]

The tocilizumab safety data was collected from five core phase 3 trials, two ongoing extension trials, and one clinical pharmacology study. A total of 4,199 patients included patients were categorized based on tocilizumab administration, and dose as follow: tocilizumab 8 mg/kg (n=1870), tocilizumab 4 mg/kg, and patients with no tocilizumab administration (n=1555). The most common adverse events and severe adverse events were an infection. The rate of infection was 112.7/100 patient-years (PY) in the tocilizumab 8-mg/kg group, 115.7/100 PY in the tocilizumab 4-mg/kg group, and 105.8/100 PY in the control group. The rate of severe adverse events was similar between three groups (tocilizumab 8 mg/kg, 14.5/100 PY; tocilizumab 4 mg/kg, 13.6/100 PY; control, 14.4/100 PY). The rate of overall adverse events was 381.6/100 PY in the tocilizumab 8-mg/kg group, 358.0/100 PY in the tocilizumab 4-mg/kg group, and 339.0/100 PY in the control group.[83] A meta-analysis of six initial trials and five long-term extensions in 601 Japanese patients, with 2188 patient-years exposure was carried out to evaluate efficacy and safety tocilizumab monotherapy in patients with moderate to severe rheumatoid arthritis. The result showed the incidence of adverse events was 465.1 per 100 PY. Furthermore, mild abnormality in lipid profile or liver function tests were common; however, none of them were categorized to be severed adverse events. In addition, the most common serious adverse event was infection (6.22 per 100 PY).[84] A systematic review and network meta-analysis of 11 RCTs and 8 cohort studies showed good cardiovascular safety of tocilizumab in comparison with other biological disease-modifying antirheumatic drugs in patients with RA. Moreover, it is

indicated that tocilizumab has a potential benefit on myocardial infarction in comparison to other biological disease-modifying antirheumatic drugs.[85]

The common adverse reactions of tocilizumab include infection, an increase of serum cholesterol, ALT, AST, and injection site reaction. Understanding tocilizumab safety and potential for toxicity in other diseases could guide clinicians to determine potential exclusion criteria in COVID-19 treatment. Tocilizumab contraindications include known hypersensitivity to tocilizumab and active infection (including localized infection). Notably, herpes zoster reactivation has been reported. Importantly, reactivation of latent tuberculosis and new infections have been observed. Before tocilizumab administration, patients should be tested concerning latent tuberculosis. In addition, it should be used with caution in patients with high risk for gastrointestinal perforation, neutropenia (absolute neutrophil count (ANC) < 2000 / mm^3: do not initiate; ANC < 500/ mm^3: discontinue), thrombocytopenia (platelet count < 100,000/mm^3: do not initiate treatment; platelet count < 50,000/mm3: discontinue treatment), hepatic impairment, demyelinating disorders, and hyperlipidemia.[82-85] Notably, patients with neutropenia (<500/mm^3), thrombocytopenia (<50,000/mm^3), suspected or confirmed bacterial infection, or active diverticulitis or gastrointestinal tract perforation were excluded from Toniati et al. study. [72] To date, the published studies have not reported most of them as exclusion criteria; however, exclusion criteria of the COVACTA trial are as follow: patients with known severe allergic reactions to tocilizumab or other monoclonal antibodies, active tuberculosis infection, suspected active infection other than COVID-19, receiving other immunomodulatory drugs with the past 3 months, pregnancy, lactation, platelet count < 50,000/mm^3, ANC < 1000/mm^3, ALT or AST > ten-times upper limit normal, and participating in other drug clinical trials within 30 days or 5 half-live of the investigational drug. Notably, according to the Massachusetts General Hospital COVID-19 treatment guideline, patients with suspected tuberculosis, including foreign-born patients from

resource-limited countries, homelessness should be tested before tocilizumab administration. Moreover, suspected patients for strongyloides should be treated empirically with ivermectin, if receiving tocilizumab. Furthermore, IL-6 serum level should be measured before the tocilizumab administration. Besides, the Italian guidelines recommended measurement of CRP, D-dimer, and ferritin with or without IL-6 levels before, and after each administration. Finally, exclusion criteria include hypersensitivity to tocilizumab or its excipients, concomitant immunomodulators or anti-rejection drugs, active infections, platelets <50.000 / mm^3, neutrophils <500 /mm^3, ALT or AST > 5 times the upper limit of the normality, bowel diverticulitis or perforation, and other contraindications of tocilizumab. [76-79]

It is important to mention that IL-6 elevation is not an accurate indicator to reflect its functional downstream effects. CRP as a marker of IL-6 bioactivity is synthesized through IL-6-dependent hepatic biosynthesis.[84] Tocilizumab administration increases the serum levels of soluble IL-6 receptor due to the longer elimination half-life of tocilizumab with soluble IL-6 receptor immune complex compared with the soluble IL-6 receptor. In addition, IL-6 level increase after tocilizumab administration through inhibition of IL-6 consumption. Importantly, it is indicated that the administration of tocilizumab does not increase the production of IL-6. According to the published data in COVID-19, regardless of clinical outcome, the administration of tocilizumab is associated with CRP serum levels reduction. Conversely, after the administration of tocilizumab, a mild increase of IL-6 serum level was observed in all patients followed by a dramatic increase in patients with disease aggravation or mortality (Table 3).

The disease-related conditions in COVID-19, clinical condition of the patient, and treatment duration may influence the incidence of adverse events. A retrospective analysis of patients with CAR T-cell induced CRS showed no adverse events with tocilizumab, suggesting its safety in both adults and pediatrics.[86] Notably, tocilizumab adverse events were assessed on

patients with different demographic and clinical characteristics. As an example, patients in CAR T-cell induced CRS trials were younger than sever COVID-19. To date, no well-designed clinical trial has been published regarding tocilizumab safety in patients with COVID-19. In the case series by Toniati and colleagues, three severe adverse events were observed during the 10-day follow-up. Among them, two patients died due to septic shock, and one experienced gastrointestinal perforation. In the study by Sciascia et al, the safety of tocilizumab was evaluated as the primary endpoint in patients with severe COVID-19, and no moderate to severe adverse events related to the drug were reported. Also, no adverse reactions were reported in other studies. [68, 70-73] Finally, drug interactions of tocilizumab should be considered. It may enhance the effects of other immunosuppressants. Importantly, tocilizumab is a cytochrome P450 enzyme inducer and may decrease the serum concentration of cytochrome P450 3A4 substrates. Consequently, it is recommended that rivaroxaban and apixaban should not be used in those receiving tocilizumab. Besides, dose adjustments of warfarin is also recommended in these patients. Notably, warfarin is primary metabolized via cytochrome P450 2C9. The proposed mechanism of action of drug interactions is the effect on decreasing the effect of IL-6 and thus an upregulation of transporters and drug metabolism enzymes. [87-92] Taken together, according to the limited data, one- or two-times administration of both intravenous and subcutaneous tocilizumab in the mentioned doses is considered to be safe in patients with severe/critical COVID-19; however, the tocilizumab safety data in other conditions should be considered in determining exclusion criteria in the future clinical trials and practice.

Limitation

This review may include some limitations. First, the treatment of COVID-19 is fluid with the discovery of new knowledge about disease-modifying treatment plans. Next, we reference

some documents in pre-print status that are not peer-reviewed at the time of writing this manuscript. So, caution should be used in referencing these documents until the publication.

Conclusion

To date, data about the use of tocilizumab in the treatment of acute lung injury in patients with COVID-19 are very limited to conclude. More large and well-designed randomized clinical trials still are needed to confirm the efficacy and safety of tocilizumab in patients with COVID-19 developed ARDS. Besides, Future studies are recommended to provide a score for determining tocilizumab indication based on the disease severity, burden of lung injury, presence of risk factors, and levels of inflammatory markers importantly IL-6. It would help clinicians to find out which population benefits more from the drug. At this time because of a lack of data, until the determination of results of ongoing clinical trials, clinical considerations about the use of tocilizumab in patients with COVID-19 should be taken in terms of patient selection, treatment dose, combination with other therapies, and safety issues.

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References

1. Wang L, Wang Y, Ye D, et al. Review of the 2019 Novel Coronavirus (COVID-19) based on current evidence. *Int J Antimicrob Agents*. 2020 Mar 19:105948.

2. World Health Organization. Coronavirus disease 2019(COVID-19) Situation Report-144. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200426-sitrep-97-covid-19.pdf?sfvrsn=d1c3e800_6.

3. WHO Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200612-covid-19-sitrep-144.pdf?sfvrsn=66ff9f4f_2.

4. Chan JF-W, Yuan S, Kok K-H, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet*. 2020 Feb 15;395(10223):514-523.

5. EL Anderson , Turnham P, Griffin JR, et al. Consideration of the Aerosol Transmission for COVID-19 and Public Health. *Risk Anal*. 2020 May;40(5):902-907.

6. Leiwen Fu, Bingyi Wang, Tanwei Yuan, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: a systematic review and meta-analysis. *J Infect*. 2020 Apr 10.

7. National Center for Health Statistics, & Centers for Disease Control and Prevention.(2020). COVID-19 Data from the National Center for Health Statistics. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html.

8. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020 Feb 24. pii: S2213-2600(20)30079-5.

9. Lechien JR , Chiesa-Estomba CM, Hans S, et al. Loss of Smell and Taste in 2013 European Patients With Mild to Moderate COVID-19. *Ann Intern Med*, 2020 May 26.

10. Zhou Y, Fu B, Zheng X, et al. Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+CD16+ monocytes in sever e pulmonary syndrome patients of a new coronavirus. *BioRxiv* 2020:2020.02.12.945576.

11. Zhou Y, Fu B, Zheng X, et al. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *Natl Sci Rev*. 2020 Mar 13:nwaa041.

12. David L Porter, MDDavid G Maloney (2019). Cytokine release syndrome (CRS). *UpToDate.* Retrieved February 7, 2019

13. Sarosiek S, Shah R, Munshi NC. Review of siltuximab in the treatment of multicentric Castleman's disease. *Ther Adv Hematol*. 2016 Dec; 7(6): 360–366.

14. Wan Y, Shang J, Graham R, et al. (2020) Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. *J Virol*. 2020 Mar 17;94(7). pii: e00127-20.

15. Zhao Y, Zhao Z, Wang Y, et al. (2020) Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan COVID-19. *bioRxiv*.

16. Crackower MA, Sarao R, Oudit GY, et al. (2002) Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*, 01 Jun 2002, 417(6891):822-828.

17. Danilczyk U, Sarao R, Remy C, et al. (2006) Essential role for collectrin in renal amino acid transport. *Nature*, 2006 Dec 21;444(7122):1088-91. Epub 2006 Dec 13.

18. Gu J, Gong E, Zhang B, et al. (2005) Multiple organ infection and the pathogenesis of SARS. *J Exp Med.* 2005 Aug 1;202(3):415-24. Epub 2005 Jul 25.

19. Ding Y, He L, Zhang Q, et al. (2004) Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol*, 203:622–630.

20. Hamming I, Timens W, Bulthuis MLC, et al. (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus: a first step in understanding SARS pathogenesis. *J Pathol* 203:631–663

21. Ding Y, Wang H, Shen H, et al. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. *J Pathol*. 2003; 200: 282-289

22. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, et al. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLoS Pathog*. 10 (2014) e1004077.

23. D. Schoeman, B.C. Fielding. Coronavirus envelope protein: current knowledge. *Virol J.* 2019 May 27;16(1):69.

24. R. Channappanavar, A.R. Fehr, R. Vijay, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. C*ell Host Microbe*. 2016 Feb 10;19(2):181-93.

25. H.K. Law, C.Y. Cheung, H.Y. Ng, et al. Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. *Blood.* 2005 Oct 1;106(7):2366-74. Epub 2005 Apr 28.

26. J.M. Nicholls, L.L. Poon, K.C. Lee, et al. Lung pathology of fatal severe acute respiratory syndrome. *Lancet*, 361 (2003), pp. 1773-1778

27. Y. Zhang, J. Li, Y. Zhan, et al. Analysis of serum cytokines in patients with severe acute respiratory syndrome Infect. *Immun*., 72 (2004), pp. 4410-4415

28. J.S. Peiris, C.M. Chu, V.C. Cheng, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361 (2003) 1767–1772.

29. M.M. Leiva-Juarez, J.K. Kolls, S.E. Evans. Lung epithelial cells: therapeutically inducible effectors of antimicrobial defense. *Mucosal Immunol*, 11 (1) (2018), pp. 21-34

30. L. Knudsen, M. Ochs. The micromechanics of lung alveoli: structure and function of surfactant and tissue components. *Histochem Cell Biol.* 2018 Dec;150(6):661-676.

31. K. Brune, J. Frank, A. Schwingshackl, et al. Pulmonary epithelial barrier function: some new players and mechanisms. *Am J Physiol Lung Cell Mol Physiol*, 308 (8) (2015), pp. L731-L745

32. Binqing Fu, Xiaoling Xu, Haiming Wei. Why tocilizumab could be an effective treatment for severe COVID-19? *J Transl Med*. 2020 Apr 14;18(1):164.

33. Xu Z , Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* . 2020 Apr;8(4):420-422.

34. J Scheller, S Rose-John. Interleukin-6 and its receptor: from bench to bedside. *Med Microbiol Immunol*. 2006 Dec;195(4):173-83.

35. K. Yasukawa, T. Hirano, Y. Watanabe, et al. Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. *EMBO J*. 1987 Oct; 6(10): 2939–2945.

36. Jones BE, Maerz MD, Buckner JH. Buckner. IL-6: a cytokine at the crossroads of autoimmunity. *Curr Opin Immunol*. 2018 Dec; 55:9-14.

37. Shimabukuro-Vornhagen A, Godel P, Subklewe M, al. IL-6: regulator of Treg/Th17 balance. *Eur J Immunol*. 2010 Jul;40(7):1830-5.

38. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*, 441 (7090) (2006), pp. 235-238

39. Christopher A. Hunter, Simon A. Jones. IL-6 as a keystone cytokine in health and disease. *Nature immunology*, 16 (5) (2015), pp. 448-457

40. Shimabukuro-Vornhagen A, Gödel P, Subklewe M, et al. Cytokine release syndrome. *J Immunother Cancer*. 2018 Jun 15;6(1):56

41. M. Norelli, B. Camisa, G. Barbiera, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med*. 2018 Jun;24(6):739-748.

42. J.R. Teijaro. Cytokine storms in infectious diseases Seminars in immunopathology. *Semin Immunopathol*. 2017; 39(5): 501–503.

43. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis Seminars in immunopathology. *Semin Immunopathol*. 2017 Jul;39(5):517-528.

44. Maude SL, Teachey DT, Porter DL, et al. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood*. 2015 Jun 25;125(26):4017-23.

45. Wang Z, Han W. Biomarkers of cytokine release syndrome and neurotoxicity related to CAR-T cell therapy. *Biomark Res*. 2018 Jan 22;6:4.

46. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013 Apr 18;368(16):1509-1518.

47. Porter DL, Levine BL, Kalos M, et al. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011 Aug 25;365(8):725-33.

48. Singh N, Hofmann TJ, Gershenson Z, et al. Monocyte lineage-derived IL-6 does not affect chimeric antigen receptor T-cell function. *Cytotherapy*. 2017 Jul;19(7):867-880.

49. Kotch C, Barrett D, Teachey DT. Tocilizumab for the treatment of chimeric antigen receptor T cell-induced cytokine release syndrome. *Expert Rev Clin Immunol*. 2019 Aug;15(8):813-822.

50. Chen H, Wang F, Zhang P, et al. Management of cytokine release syndrome related to CAR-T cell therapy. *Front Med*. 2019 Oct;13(5):610-617

51. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. 2020 Mar 28;395(10229):1033-1034.

52. Stebbing J, Phelan A, Griffin I, et al. COVID-19: combining antiviral and anti-inflammatory treatments. *Lancet Infect Dis*. 2020 Apr;20(4):400-402.

53. Wang W, He J, Lie p, et al. (2020) The definition and risks of Cytokine Release Syndrome-Like in 11 COVID-19-Infected Pneumonia critically ill patients: Disease Characteristics and Retrospective Analysis. *medrRxiv*

54. Chen L, Liu HG, Liu W, et al. Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi.* 2020 Feb 6;43(0):E005.

55. Ruan Q, Yang K, Wang W, et al. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Medicine*, 2020 Mar 3 : 1–3.

56. F. Zhou, T. Yu, R. Du, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A Retrospective Cohort Study. *Lancet* 395 (2020) 1054–1062.

57. Ledford H. How Does COVID-19 Kill? Uncertainty Is Hampering Doctors' Ability to Choose Treatments. *Nature*. 2020 Apr;580(7803):311-312.

58. NIH. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. https://covid19treatmentguidelines.nih.gov/introduction/.

59. COVID19 Treatment Guidelines by Massachusetts General Hospital. https://medtube.net/infectious-diseases/medical-documents/26086-covid19-treatment-guidelines-by-massachusetts-general-hospital.

60. Li H, Chen C, Hu F, et al. Impact of corticosteroid therapy on outcomes of persons with SARSCoV-2, SARS-CoV, or MERS-CoV infection: a systematic review and meta-analysis. *Leukemia*. 2020

61. Fadel R, Morrison, A. R, Vahia A, et al. Short Course Corticosteroids in Hospitalized Patients with COVID-19. *Clinical Infectious Diseases,* ciaa60. 19 May 2020.

62. A Fernandez-Cruz, B Ruiz-Antoran, A Sancho-Lopez, et al. IMPACT OF GLUCOCORTICOID TREATMENT IN SARS-COV-2 INFECTION MORTALITY: A RETROSPECTIVE CONTROLLED COHORT STUDY (2020), medRxiv.

63. Shakoory B, Carcillo JA, Chatham WW, et al. Interleukin-1 Receptor Blockade Is Associated With Reduced Mortality in Sepsis Patients With Features of Macrophage

Activation Syndrome: Reanalysis of a Prior Phase III Trial. *Crit Care Med*. 2016 Feb; 44(2):275-81.

64. F. Zhou, T. Yu, R. Du, et al., Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A Retrospective Cohort Study. *Lancet* ,395 (2020) 1054–1062.

65. Stanislas Bataille, Nathalie Pedinielli, Jean-Philippe Bergougnioux. Could ferritin help the screening for COVID-19 in hemodialysis patients? *Kidney Int*. 2020 Apr 22

.

66. Zhang X, Song K, Tong F, et al. First case of COVID-19 in a patient with multiple myeloma successfully treated with tocilizumab. *Blood Adv,* (2020) 4 (7): 1307–1310.

67. J-M Michot, L Albiges, N Chaput, et al. Tocilizumab, an anti-IL6 receptor antibody, to treat Covid-19-related respiratory failure: a case report. *Annals of Oncology*, 27 March 2020.

68. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. *ChinaXiv*. March 2020. http://chinaxiv.org/abs/202003.00026.

69. L Lin, T S Li. [interpretation of "guidelines for the diagnosis and treatment of novel coronavirus (2019-ncov) infection by the national health commission (trial version 5)"]. *Zhonghua Yi Xue Za Zhi.* 2020 Feb 7;100(0):E001.

70. Pan Luo, Yi Liu, Lin Qiu, et al. Tocilizumab treatment in COVID- 19: A single center experience. *J Med Virol.* 2020 Apr 6;10.1002/jmv.25801.

71. Sciascia S, Apra F, Baffa A, et al. Pilot Prospective Open, Single-Arm Multicentre Study on Off-Label Use of Tocilizumab in Patients With Severe COVID-19. *Clin Exp Rheumatol* . May-Jun 2020;38(3):529-532. Epub 2020 May 1.

72. Toniati P, Piva S, Cattalini M, et al. Tocilizumab for the Treatment of Severe COVID-19 Pneumonia With Hyperinflammatory Syndrome and Acute Respiratory Failure: A Single Center Study of 100 Patients in Brescia, Italy. *Autoimmun Rev*. 2020 Jul;19(7):102568.

73. Moreno-García E, Rico V, Albiach L. Tocilizumab is associated with reduced risk of ICU admission and mortality in patients with SARS-CoV-2 infection. (2020). *medRxiv*.

74. V Jain, JM Yuan. Systematic review and meta-analysis of predictive symptoms and comorbidities for severe COVID-19 infection (2020), *medRxiv*.

75. National Health Committee of the People's Republic of China. China's National Health Commission treatment guidelines 7th version [Internet]. Beijing; 2020 [cited 2020 Mar16]. http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml

76. A Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia (COVACTA). https://clinicaltrials.gov/ct2/show/NCT04320615

77. Buonaguro FM, Puzanov I, Ascierto PA. Anti-IL6R role in treatment of COVID-19-related ARDS. *J Transl Med*. 2020 Apr 14;18(1):165.

78. Vidant Health Tocilizumab and COVID-19. https://www.vidanthealth.com/getattachment/COVID-19-Updates/For-Physicians/Tocilizumab-and-COVID-19-v3-April-10-2020.pdf?lang=en-US

79. Tocilizumab in COVID-19 Pneumonia (TOCIVID-19) (TOCIVID-19). https://clinicaltrials.gov/ct2/show/NCT04317092.

80. Zhong J, Tang J, Ye C. The immunology of COVID-19: is immune modulation an option for treatment? *Lancet Rheumatol.* May 20, 2020.

81. YR Ren, Golding A, Sorbello A. A Comprehensive Updated Review on SARS-CoV-2 and COVID-19. *J. Clin. Pharmacol*. May 29, 2020.

82. Smolen JS, Beaulieu A, Rubbert-Roth A, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet*. 2008 Mar 22;371(9617):987-97.

83. MH Schiff, JM Kremer , A Jahreis, et al. Integrated safety in tocilizumab clinical trials. *Arthritis Research & Therapy* volume 13, Article number: R141 (2011)

84. Nishimoto N, Ito K, Takagi N. Safety and efficacy profiles of tocilizumab monotherapy in Japanese patients with rheumatoid arthritis: meta-analysis of six initial trials and five long-term extensions. *Modern Rheumatology* volume 20, pages222–232(2010) Published: 11 March 2010.

85. Castagne´ B, Viprey M, Martin J, et al (2019). Cardiovascular safety of tocilizumab: A systematic review and network meta-analysis. *PLoS ONE* 14(8): e022017885.

Le RQ, Li L, Yuan W, et al. FDA Approval Summary: Tocilizumab for Treatment of Chimeric Antigen Receptor T Cell-Induced Severe or Life-Threatening Cytokine Release Syndrome. *Oncologist*, 2018 Aug; 23(8): 943–947.

86. N.M. Noda-Nicolau, J. Polettini, M.G. da Silva, et al. Polybacterial stimulation suggests discrete IL-6/IL-6R signaling in human fetal membranes: potential implications on IL-6 bioactivity. *J. Reprod. Immunol*. 126 (2018) 60–68.

87. Weitz JI, Raskob GE, Spyropoulos AC, et al. Thromboprophylaxis with Rivaroxaban in Acutely Ill Medical Patients with Renal Impairment: Insights from the MAGELLAN and MARINER Trials. *Thromb Haemost,* 2020; 120(03): 515-524.

88. Spyropoulos AC, Lipardi C, Xu J, et al. Modified IMPROVE VTE Risk Score and Elevated D-Dimer Identify a High Venous Thromboembolism Risk in Acutely Ill Medical Population for Extended Thromboprophylaxis. *TH Open*, 2020; 04(01): e59-e65.

89. American Society of Hematology Antithrombotic Therapy in Patients with COVID-19, Last Updated: May 12, 2020. https://www.covid19treatmentguidelines.nih.gov/antithrombotic-therapy/.

90. B Bikdeli, MV Madhavan, D Jimenez, et al. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J. Am. Coll. Cardiol*, 2020, Vol 75, Issue 23, p. 2950-2973.

91. Morgan ET, Goralski KB, Piquette-Miller M, et al. Regulation of Drug-Metabolizing Enzymes and Transporters in Infection, Inflammation, and Cancer. *Drug Metab Dispos*. 2008 Feb;36(2):205-16.

92. Shah RR. Smith RL. Inflammation-induced Phenoconversion of Polymorphic Drug Metabolizing Enzymes: Hypothesis With Implications for Personalized Medicine. *Drug Metab Dispos*. 2015 Mar;43(3):400-10.

Legend

Fig1. Interleukin 6 Role in Chimeric Antigen Receptor T cells Induced Cytokine Release Syndrome

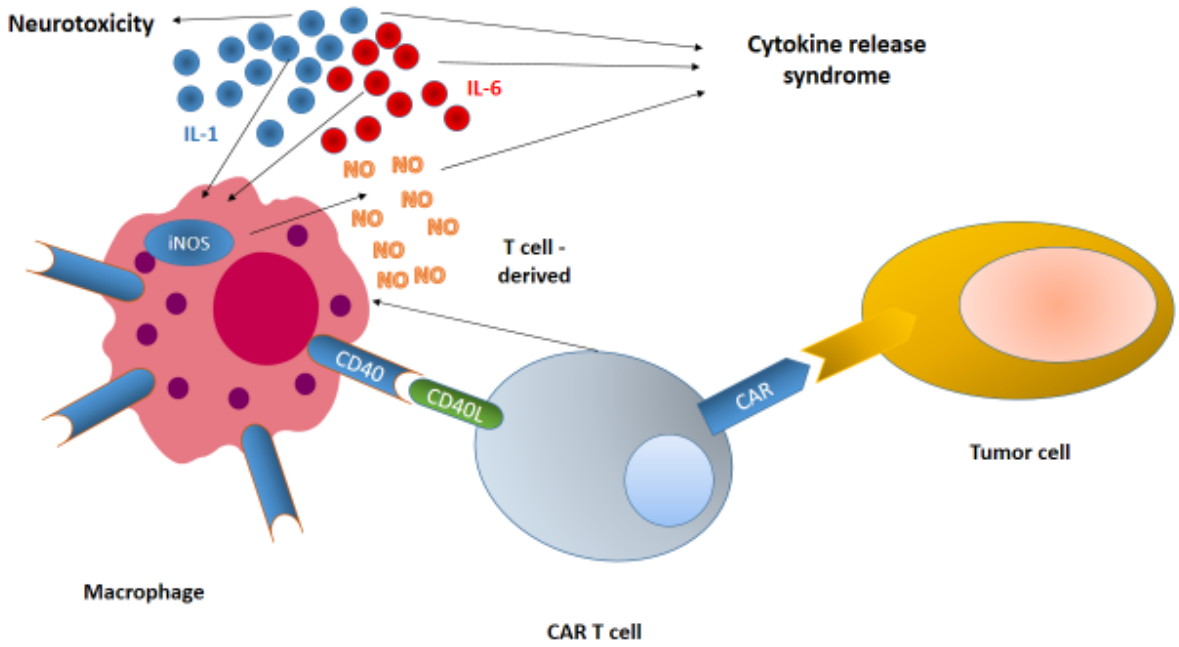

Fig1.

Author, Year	Design	Country	Population (n=sample size)	Tocilizumab dose	Other treatments	Follow-up, days	Outcomes
Zhang et al, 2020	Case report	China	Respiratory failure (n=1)	IV; 8mg/kg; two doses	Lopinavir-Ritonavir	42	Fever resolution; DC of oxygen supplementation; radiological improvement in ground glass changes reduction from 225mg/L to 33mg/L
Michot et al, 2020	Case report	France	Respiratory failure (n=1)	IV; 8mg/kg;	Methylprednisolone, IV, 5 days	15	Chest symptoms resolution; reduction of IL-6 levels to normal
Xu et al, 2020	Retrospective, single-center, case series	China	Severe or critical (n=21)	IV; 400mg; one or two doses, 12 hours interval	Lopinavir Methylprednisolone	NR	Fever resolution (100%); reduction of oxygen support (75%); radiological improvement (91%); lymphocytes return to normal (53%) CRP returning to normal (84%) 91% Discharged; 9% remain stable
Luo et al, 2020	Retrospective, single-center, case series	China	Moderate, severe or Critical (n=15)	IV; 80- 600 mg. 33% administered subsequent doses	Methylprednisolone in 53% of patients	7	Death (20%); disease worsening (13%), clinical stability (67%), CRP reduced from 126.9 to 11.2 mg/L. Drop in IL-6 (67%)
Sciascia et al, 2020	prospective, multi-center, case series	Italy	Severe COVID-19 (n = 63)	IV; 8 mg/kg or SC; 324 mg, one or two doses, 24 hours interval	Lopinavir/ritonavir in 71.4% of patients; Darunavir/cobicista in 28.6%	14	No moderate-to severe adverse events related to tocilizumab. Significant improvement in ferritin, CRP, D-dimer, PaO2/FiO2 ratio. Increase of likelihood of survival within 6 days Improving or stabilizing clinical condition 77% of patients, worsening in 23% (20%died).
Toniati et al, 2020	prospective, single center, case series	Italy	Severe COVID-19 (n = 100)	IV: 8 mg/kg, two doses, 12 hours interval; third dose is based on clinical response, one day interval.	Lopinavir, ritonavir or remdesivir + hydroxychloroquine + dexamethasone+ AB prophylaxis		Lymphocyte count, CRP, fibrinogen and ferritin serum levels improved. Tocilizuamb adverse effects: Septic shock (2%), GI perforation (1%).

				IV: 400 to 600 mg(based on weight), one to three dose based on response to treatment (in 77 patients)		10	Reduction in ICU admissions and mechanical ventilation use, lower mortality (10.3%) than other reports.
Moreno-García et al, 2020	Retrospective, single center, non-randomized study	Spain	Non-critically ill COVID-19 patients (171)		Antiviral drugs, HCQ, azithromycin, LWMH (if risk factors for thrombosis), methylprednisolone (if disease progression to ARDS)	N/A	

Table1. Published Clinical Trials Investigating the Therapeutic Effect of Tocilizumab for the Treatment of COVID-19
IV indicates intravenous; DC, discontinuation; CRP, C - reactive protein; IL, interleukin; SC, subcutaneous; PaO2/FiO2, atrial partial pressure of oxygen / fraction of inspiration O2, AB, antibiotic; GI, gastrointestinal; HCQ, hydroxychloroquine; LWMH, low molecular weight heparin; ARDS, acute respiratory distress syndrome.

Table2. Summary of Ongoing Clinical Trials Investigating the Therapeutic Effect of Tocilizumab for the Treatment of COVID-19

ID	Status	Design	Country	Population (n patients)	Intervention Group(s)	Comparison group(s)	Primary Outcomes
NCT04317092	Recruiting	Multicenter, single-arm, open-label, clinical trial	Italy	COVID-19 Pneumonia (400)	Tocilizumab 8 mg/kg (up to 800mg per dose) IV, with an interval of 12 hours.	No comparison group	One-month mortality rate
NCT04345445	Not recruiting	Open-label, Randomized, Cross-over clinical trial	Malaysia	COVID-19 (n= 310)	Tocilizumab 8 mg/kg IV once	Methylprednisolone 120mg/day for 3 days	Requiring mechanical ventilation, Mean days of ventilation
NCT04331795	Recruiting	Single Group, clinical trial	United States	Hospitalized, non-critically ill patients with COVID-19 pneumonia (n=50)	Tocilizumab low dose 80 mg or 200mg IV (based on risk factors for decompensation), up to two doses within 24 hours based on response	No comparison group	Clinical response, Biochemical response
NCT04332094	Recruiting	Randomized, Multicenter, Open-label Clinical Trial	Spain	COVID-19 (n = 276)	Hydroxychloroquine plus Azithromycin plus Tocilizumab 162 mg SC x 2 doses plus Tocilizumab 162mg SC x 2 doses at 12 hours (day 1) Standard care plus Tocilizumab 8 mg/kg IV up to a maximum of 800 mg with repetition of the same dosage after 12 hours	Hydroxychloroquine plus Azithromycin	In-hospital mortality , Need for mechanical ventilation in the Intensive Care
NCT04346355	Recruiting	Open-label Randomized Multicenter clinical trial multicenter, double-blind, randomized controlled clinical trial	Italy	COVID-19 Pneumonia (n=398) SARS-CoV-	Tocilizumab 8 mg/kg IV after confirmation of progressive dyspnea. Repeated once if no improvement in the 8-point WHO scale Tocilizumab 8 mg/kg IV, An additional	Standard of care 100 mL NaCl 0.9% after	Entry into Intensive Care with invasive mechanical ventilation or death

				2 Infection (n= 100)	dose may be given if clinical symptoms worsen or show no improvement.	confirmation of progressive dyspnea	ICU admission or intubation or death
NCT04335071	Not recruiting	Randomized, Double-Blind, Placebo-Controlled, Multicenter	Switzerland	Sever COVID-19 Pneumonia (n=330)	Tocilizumab 400 mg IV in a single dose, with a possible second dose in case of no clinical response	Placebo	Clinical Status Assessed Using a 7-Category Ordinal Scale
		Observational			Tocilizumab 8 mg/kg IV once in 100 ml 0.9% saline IV		
NCT04320615	Recruiting		United States	COVID-19 Respiratory Distress Syndrome and Cytokine Release Syndrome (n=30)		No comparison group	Complete recovery defined as fever disappearance and return to normal peripheral oxygen saturation values
NCT04332913	Recruiting	Observational	Italy	COVID-19 Cytokine Release Syndrome(n=120)	Tocilizumab 4 to 8 mg/kg IV once. an additional dose may be given if there is fever after 12 hours alone or with Favipiravir Tocilizumab 8mg/kg. If no response (no decrease of oxygen requirement) a second injection after 2 days.	Continuous renal replacement therapy	Normalization of Fever and Oxygen Saturation
NCT04306705	Recruiting	Three arms, Multicenter, Randomized Controlled Trial	China	COVID-19 (n=150)	Tocilizumab (400 mg), IV Tocilizumab, (2 x 162 mg), SC Sarilumab (200 mg), SC	Favipiravir alone	Clinical cure (Viral negative load, lung image improvement, clinical manifestation)
		Multiple Randomized Controlled Trials				Standard care	Survival without needs

NCT04310228	Recruiting		China	Moderate or severe COVID-19 pneumonia (n= 240)	Tocilizumab 8mg/Kg single intravenous administration		of ventilator utilization, WHO progression scale <=5, Cumulative incidence of successful tracheal extubating
NCT04331808	Not recruiting	Randomized, Factorial Design, clinical trial	France	COVID-19 Acute Hypoxic Respiratory Failure and Systemic Cytokine Release Syndrome(n=342)	Chloroquine analog nivolumab tocilizumab 400 mg	Usual Care	Time to clinical improvement
NCT04322773	Recruiting	Single Group clinical trial	Belgium	COVID-19 Severe Pneumonitis (n=38)	In case of diagnosis of MAS, IV anakinra 200mg In case of diagnosis of immune dysregulation tocilizumab 8mg/kg once up to a max of 800mg	No comparison group	Arrest in deterioration of pulmonary function, improving in pulmonary function
		Controlled, randomized, multicenter clinical trial		Advanced or Metastatic Cancer and COVID-19 Infection (n= 273)	Tocilizumab 8 mg/kg ,an addition dose based on respiratory function after 12 hours plus pembrolizumab	Standard Care	28-day survival rate
NCT04315480	Active, not recruiting	Factorial Assignment	Italy	(COVID-19) associated with organ dysfunction(n=20)	Tocilizumab 400 mg IV plus Standard Care		Change of SOFA score, Improvement of lung involvement measurements, Increase of pO2/FiO2 ratio
					Hydroxychloroquine plus Oseltamivir 75 plus Lopinavir-Ritonavir plus	No comparison group	
	Recruiting	Multicenter, randomized, controlled, open-label, phase 2 clinical		COVID-19-Related Mild Acute Respiratory Syndrome Nonresponsive to Frontline Therapy (n=24)	Tocilizumab 400 mg I.V infusion as a single dose		Normalization of SpO2 ≥96%

		trial	France				
NCT04333914		Clinical trial		COVID-19 with P/F<300 Negative IGRA test IL-6>7 (n=100)	Conventional therapy plus Tocilizumab (4-8 mg/kg) IV or (two or three injections of 162 mg plus the standard treatment. Inadequate response: administered with a 12-hour interval between injections.	Standard Care	Oxygenation status, complications in vital organs, hemodynamic disturbances, duration of mechanical ventilation, mortality
NCT04339712	Recruiting	Non-controlled clinical trial	Greece	COVID-19 with Respiratory rate > 30/min Oxygen saturation < 90% PaO2/FiO2 < 300mmHg, High level of IL-6 (n=40)	conventional therapy + tocilizumab	Standard Care	Fever, Cough, Dyspnea
NCT04335305	Recruiting	phase III, open-label, and single-arm study	Spain	COVID-19 with SpO2 ≤%93, high IL-6 (n=500)	Tocilizumab plus conventional therapy	No comparison group	Normalization of fever and oxygen saturation within 14 days of treatment
IRCT2020040604 6968N1	Recruiting		Iran	COVID-19 pneumonia with high IL-6 (n = 188)	Tocilizumab, IV immunoglobulin, and CRRT	No comparison group	
IRCT20151227025726N13	Recruiting	Multi-center randomized controlled trial	Iran	Severe COVID-19 pneumonia with high IL-6 , and grade 2-3 cytokine-release syndrome (n = 60)	Favipiravir Combined With Tocilizumab 4 to 8mg/kg. For fever patients, an additional application is given if there is still fever within 24 hours, the interval between two medications ≥ 12 hours		Cure rate
		Multi-center		Severe COVID-19 pneumonia	Tocilizumab (1-8		

		nonrandomized open label intervention		(n=100)	mg/kg) up to 800 mg per dose, can be repeated once after 12 hours plus standard treatment	Resolution of cytokine release syndrome
					Conventional therapy	
				COVID-19 pneumonia(n=150)		
	Recruiting	Single-Centre Non-randomized intervention	Iran			
IRCT20150303021315N17						Duration of hospitalization
					No comparison group	
		Multicenter, Randomized, Controlled Trial				
						Clinical cure rate
				Severe Pneumonia (n = 30)		
					No comparison group	
	Recruiting					
		Prospective Single Center Study	China			
ChiCTR2000029765					No comparison group	
						IL-6 and sIL-6R levels during 28 days
	Not yet recruiting		China			
ChiCTR2000030196						
					No comparison group	
	Cancelled by Investigator		China			

ChiCTR2000030442	Recruiting	China
ChiCTR2000030894	Recruiting	Croatia
NCT04359667		

COVID-19, coronavirus disease 2019; IV, intravenous; SC, subcutaneous; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ICU, intensive care unit; WHO, word health organization; MAS, macrophage activation syndrome; SOFA, Sequential Organ Failure Assessment, PaO2/FiO2; atrial partial pressure of oxygen / fraction of inspiration O2, IGRA; Interferon Gamma Release Assay, IL; interleukin, CRRT; continuous renal replacement therapy, sIL-6R; Soluble Interleukin-6 receptor.

Table3. CRP, and IL-6 levels in Published Clinical Trials Investigating the Therapeutic Effect of Tocilizumab for the Treatment of COVID-19

Author, year, sample size	IL-6 follow-up (day)	IL-6 before therapy (pg/mL), mean ± SEM	Il-6 after therapy (pg/mL), mean ± SEM	IL-6 changes (pg/mL), mean ± SEM	CRP follow-up (day)	CRP before therapy (mg/L), mean ± SD	CRP after therapy (mg/L), mean ± SD	CRP changes (mg/L), mean ± SEM
Xu et al, 2020, 21	NA	NA	NA	NA	5 (5 - 5)	75.1 ± 14.5	2.7 ± 0.7	-72.3 ± 13.8
Luo et al, 2020, 15	7 (3-7)	111.0 ± 43.8	1228.0 ± 470.3	1117 ± 426.5	7 (4 - 7	131.8 ± 19.9	16.8 ± 6.9	-115 ± 13
Sciascia et al, 2020, 63	14 (6-14)	105 ± 15	1125 ± 245	1150 ± 230	14 (6-14)	135 ± 15	13 ± 3	-123 ± 12
Toniati et al, 2020, 100	10 [a]	41 (10-102) [b]	1812 (375 - 2600)	1771 (365 - 2498)	10	113 (45 - 169)	2 (1 – 5)	-111 (44 – 164)

IL indicates interleukin; CRP, C - reactive protein; SD, standard deviation. Follow-up days present as median (range) and have been estimated from Sciascia et al results. IL-6, and CRP values have been estimated from Sciascia et al Figures 1, and 2.

[a] follow-up days range was not available for Toniati et al.

[b] IL-6 and CRP serum levels data have been expressed as median (1st Quartile - 3rd Quartile).